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Assessment of the Microscreen Phage-Induction Assay for Screening Hazardous Wastes (1989)

(U.S.) Health Effects Research Lab., Research Triangle Park, NC

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ASSESSMENT OF THE MICROSCREEN PHAGE-INDUCTION ASSAY FOR SCREENING HAZARDOUS WASTES*

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ABSTRACT: The Microscreen phage-induction assay, which quantitatively measures the induction of prophage λ in Escherichia coli WP2s(λ), was used to test 14 crude (unfractionated) hazardous industrial waste samples for genotoxic activity in the presence and absence of metabolic activation. Eleven of the 14 wastes induced prophage, and induction was observed at concentrations as low as 0.4 picograms per ml. Comparisons of the mutagenic activity of these waste samples in Salmonella and their ability to induce prophage λ indicate that the phage-induction assay was a more sensitive indicator of genetic damage for this group of wastes. All but one of the wastes that were mutagenic to Salmonella were detected by the phage-induction assay, and 5 wastes not mutagenic to Salmonella were genetically active in the phage assay. The enhanced ability of the phage-induction assay to detect genotoxic activity may be related to the constituents comprising these waste samples. Partial chemical characterizations of the wastes showed high concentrations of carcinogenic metals, solvents, and chlorinated compounds, most of which are detected poorly by the Salmonella assay. However, recent studies of the induction of prophage by these chemical classes have suggested that phage induction may be a sensitive endpoint for these groups of chemicals.

KEYWORDS: prophage induction, Microscreen assay, hazardous industrial wastes, genotoxicity

*Data presented in this paper have been published previously [1].

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According to estimates by the U.S. Environmental Protection Agency (US EPA), more than 260 million metric tons of hazardous wastes are generated annually, a quantity equal to more than 70 billion gallons [2]. Some of the acute health effects linked to hazardous waste exposures include lung and skin irritations [3], eye irritations and menstrual problems [4], and transitory liver damage [5,6]. Included among the chronic health effects associated with hazardous waste exposure are chromosomal damage, cancer, and reproductive anomalies [7-9].

One of the ways by which wastes are classified as hazardous is on the basis of specific chemical constituents that may be present in the waste [10,11]. However, analyzing a waste for a limited set of compounds may not provide data that reflect the overall chemical composition of the waste, nor does it take into account the possibility of chemical interactions (antagonisms, synergisms, etc.) or the production of metabolites resulting from degradative pathways. In order to provide more useful information for determining the human health effects that may be caused by a hazardous waste, toxicological assessments of the waste should be made.

Because of the presence of known mutagens and carcinogens in many industrial wastes, genetic toxicity represents an important biological response. Genotoxicity as a biological endpoint may be measured in a number of ways. For example, the Salmonella assay developed by Dr. Bruce Ames and coworkers [12] has been used more than any other short-term assay for determining the genotoxicity of various complex environmental mixtures, including hazardous wastes. However, because hazardous wastes can contain high concentrations of carcinogenic metals, chlorinated compounds, and solvents [13] that are detected poorly by the Salmonella assay [14-16], the genotoxic potential of some wastes may escape detection when evaluated exclusively by the Salmonella assay.

An assay that can detect the genotoxic activity of a wide variety of chemical classes, including carcinogenic metals [17], chlorinated compounds [18], and solvents [19], is the Microscreen phage-induction assay developed by Rossman *et al*. [17]. It is a rapid and inexpensive assay that quantitatively measures the induction of prophage λ in *Escherichia coli* $WP2_s(\lambda)$. Prophage induction is one of several events that may occur upon induction of the SOS response, which is a cellular reaction to DNA damage. Other SOS functions include DNA repair, transformation, and mutagenesis. Many agents that induce the SOS response in bacteria are also genotoxic in a variety of other organisms [20]. Thus, because prophage induction can be caused by a variety of genetic mechanisms and by a wide range of genotoxic chemicals (including some not detected in the Salmonella assay), we have used prophage induction as an endpoint to determine the genotoxicity of several hazardous waste samples.

EXPERIMENTAL METHOD

Fourteen crude hazardous waste samples were obtained from Edward L.

Kanz, Hazardous Waste Engineering Laboratory, US EPA, Cincinnati, OH (Table 1). Three of the waste samples were from petrochemical, pharmaceutical, and plastics manufacturers (A, B, and C, respectively). The remaining wastes (D through O) were composites of wastes from a variety of industrial sources that were collected by 4 commercial hazardous waste incineration facilities and classified according to their aqueous or organic properties. Table 1 provides a cursory physical description of the wastes, all of which were liquids or semi-solids.

TABLE 1 -- Physical descriptions of hazardous wastes

Waste	Physical State	Description
A	Liquid	Black, very thin oil from a petrochemical manufacturing plant
B	Liquid	Black, oily liquid from a pharmaceutical manufacturer
C	Tar	Black, pourable tar produced during the production of plastics
D	Liquid	Composite of aqueous wastes; watery liquid with red oil droplets
F and G	Semi-solid	Composite of organic wastes; biphasic gray sludge with reddish-brown liquid
H and I	Suspension	Composite of aqueous wastes; thin, gray slurry
J	Liquid	Composite of organic wastes; gray, thick liquid with suspended solids
K	Liquid	Similar to K, but lighter in color and thinner
L and M	Tar	Composite of organic wastes; black, thin, pourable tar
N and O	Liquid	Composite of aqueous wastes; clear, watery liquid

Table 2 shows the results of a partial chemical analysis that was performed on these samples [13]. These characterizations should not be viewed as indicative of the overall chemical composition of the wastes because the analysis was limited to specific organics and/or metals identified in the EPA Appendix VIII list of priority pollutants. In addition, for purposes of a previous study on the performance of hazardous waste incinerators [13], 7 of the waste samples (B, F, G, J, K, L, and M) were spiked with carbon tetrachloride and trichloroethylene. Thus, the concentrations of these 2 chemicals in these 7 waste samples reflect this addition.

Test Procedure

The phage-induction assay was performed as described by Rossman *et al*. [17] with modifications [1,18]. The bacterial strains are derivatives of *E. coli* B/r. WP2s(λ) is a lambda lysogen of WP2s (*trpE*, *uvrA*), and SR714 (*trpE*, *uvrD3*) is the indicator strain. The crude (unfractionated) waste samples were serially diluted in supplemented minimal medium in sterile 96-well microtiter dishes (Corning). The lysogenic strain of *E. coli* was then exposed overnight (20 h, 37°C) to

TABLE 2 -- Concentrations of chemicals and metals identified in hazardous wastes (μg/g)

Chemical/Metal[a]	Petro-Chemical	Pharma-ceutical	Plastics	Hazardous Waste Incineration Facilities									
				1	2				3		4			
				Aqueous	Organic		Aqueous		Organic		Organic		Aqueous	
	A	B	C	D	F	G	H	I	J	K	L	M	N	O
Aniline		14000	550000											
Benzyl Chloride	3000													
Bis-(2-ethyl-hexyl)-phthalate		500		< 100							200	230	< 10	< 10
Butylbenzyl-phthalate				< 100	120	450	< 5	8	160	140				
Chlordane											< 60	< 60	19000	19000
Chlorophenylisocyanate		21000												
Cresol(s)									2000	2500				
Diethylphthalate					620	1300	240	240						
m-Dichlorobenzene		23000												
o-Dichlorobenzene		46000												
p-Dichlorobenzene		59000												
m-Dinitrobenzene			< 100											
Diphenylamine			6200											
2,4-Dimethylphenol									500	2000				
Hexachlorobutadiene											< 10	< 10	< 10	< 10
Hexachloroethane	560										< 10	< 10	230	260
Hexachlorocyclo-pentadiene														
Isophorone				< 100										
Mononitrobenzene			< 100											
Naphthalene	< 100			< 100	250	450	44	49	450	490	38	33	< 10	< 10
Phenol				34000	1500	1700	1800	2900						
Phenylenediamine			2300											
Phenylisocyanate		160000												
Trans-1,4-dichloro-2-butene	59000													
1,2,4-Trichlorobenzene		290									46000	58000	< 10	< 10
Benzene				< 3							9100	11300	< 10	< 10
Carbon tetrachloride	68000	44000		< 2	3700	4400	< 10	< 10	5900	5700	390	500	< 10	< 10
Chlorobenzene		4100												

TABLE 2 -- cont'd.

Chemical/Metal[a]	Petro-Chemical	Pharma-ceutical	Plastics	Hazardous Waste Incineration Facilities									
				1	2				3		4			
				Aqueous	Organic		Aqueous		Organic		Organic		Aqueous	
	A	B	C	D	F	G	H	I	J	K	L	M	N	O
Chloromethane		1200												
Chloroform	2900				170	270	21	22			110	60	< 10	< 10
Cis-1,4-dichloro-2-butene	18000										< 10	< 10	3100	4700
Methylene Bromide											100	340	44	60
Methylene Chloride	21000								18000	38000				
Methyl Ethyl Ketone				27										
Tetrachloroethylene	11000			< 1	7100	9800	1600	1300	7900	8100	87	150	< 10	< 10
Toluene	240000			110	32000	45300	2900	2700	56000	48000	160000	390000	22	20
1,1,1-Trichloroethane	< 100								24000	16000	330	230	< 10	< 10
Trichloroethylene	4000	40000		< 1	3700	4400	90	85	8100	7800	8300	10300	< 10	< 10
Ag	< 3				< 1	< 1	< 1	< 1			< 1	< 1		
As	< 24				< 20	< 20	< 20	< 20	< 14	< 14	< 23	< 23		
Ba	< 7				110	140	6	7	1160	1150	990	1190		
Be	< 2				< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1		
Cd	< 5				6	6	< 1	< 1	153	15	49	55		
Cr	< 5				50	57	3	3	431	425	250	290		
Hg	< 22				< 10	< 10	< 10	< 10	< 4	< 4	< 50	< 50		
Ni	< 68				7	7	2	2	26	27	< 4	< 4		
Pb	< 19				140	150	< 10	11	1830	1800	1200	1300		
Sb	< 12				58	61	< 10	< 10	437	373	< 24	< 24		
Se	< 470				340	< 100	< 100	< 100	< 21	< 21	< 160	< 160		
Tl	< 23				< 20	< 20	< 20	< 20	< 9	< 9	< 22	< 22		
Si									< 1	< 1				
pH		2		8	7	7	6	7				7	7	7
Water (%)	2		5	94	38	48	95	67	21	23	5	5	94	96

[a]Data from [13]. Waste samples B,E,F,G,J,K,L, and M were spiked with carbon tetrachloride and trichloroethylene; therefore, the concentrations of these 2 chemicals in these wastes reflect this addition.

the diluted wastes in the presence and absence of an exogenous metabolic activation system (S9). Following exposure, wells of the microtiter dishes were scored for turbidity (cell survival and growth) or clarity (cytotoxicity and/or inhibition of cell growth). Turbidity in the wells of microtiter dishes was often difficult to discern due to the physical nature of these crude complex mixtures. Many of the waste samples produced a precipitate (noted by a "P" in Table 3) or an oily film (noted by an "F") that complicated the determination of whether the wells were clear or turbid. The cytotoxic responses of the waste samples are noted by a "T" for clear wells and a "t" for slightly turbid wells in Table 3. Wells of the microtiter dishes were then sampled for the presence of prophage by exposing diluted contents of selected wells to the indicator strain (SR714) and enumerating the resulting plaque-forming units (PFU).

RESULTS

The appearance of a dose-related increase of induced (observed minus background) PFU that reached or exceeded the upper limits of the 99% confidence interval for the control plates indicated a positive, or genotoxic, response. The background PFU/plate were calculated by averaging results from eight medium control wells (with and without S9) for each experiment. The upper limits of the 99% confidence intervals were 102 PFU/plate in the presence of S9 and 30 PFU/plate in the absence of S9.

The response for each waste sample based on the criterion described above is shown in Table 3, which also presents raw data from a representative experiment for each of the wastes. Reported doses were selected to illustrate the response range for each waste tested. Eleven of the 14 crude hazardous waste samples induced prophage; only 2 of the wastes (D and J) required S9 for induction. In addition to showing the induced PFU/plate, Table 3 also indicates the fold increase over the background. Most of the positive waste samples produced a 3- or 4-fold increase over the background, which is consistent with the fold increase for a positive response observed in a previous study of pure compounds [18].

Dose-response curves for the waste samples are illustrated in Figs. 1-3. Except for waste samples A and C in the presence of S9 (Fig. 3), each waste sample produced a dose-response curve exhibiting a linear portion that spanned ~1 order of magnitude of dose (Figs. 1 and 2). Based on the linear portions of these dose-response curves, we calculated the concentration required by each waste to produce an induced PFU/plate equal to the upper limits of the 99% confidence intervals of the medium controls (Table 4). Each of these values represents the minimum concentration of the waste required to produce a positive response based on our criterion described previously. The waste samples were then ranked from the most to the least potent (Table 4).

TABLE 3 -- Induction of prophage lambda by hazardous wastes

	+S9				-S9			
Waste	Dose (μg/ml)	PFU per Plate[a]	Induced PFU per Plate[b]	Fold in-crease	Dose (μg/ml)	PFU per Plate	Induced PFU per Plate	Fold in-crease
A	0	21	0	--	0	12	0	--
	5.0×10^{-5}	82	61	3.9	2.0×10^{-7}	30	18	2.5
	2.0×10^{-4}	160	139	7.6	4.0×10^{-7}	49	37	4.1
	1.2×10^{-2}	320	299	15.2	8.0×10^{-7}	60	48	5.0
	2.5×10^{-2}	388	367	18.5	1.5×10^{-6}	2(T)	0	--
	1.5	552	531	26.3				
	50	241	220	11.5				
	200	182	161	8.7				
	400	0(T)	0	0				
	Response	+				+		
B	0	21	0	--	0	12	0	--
	0.05	249	228	11.9	2.0×10^{-7}	24(P)	12	2.0
	0.1	421	400	20.0	4.0×10^{-7}	49(P)	37	4.1
	0.2	582	561	27.2	8.0×10^{-7}	106(P)	94	8.8
	0.4	957	936	45.6	1.5×10^{-6}	136(P)	124	11.8
	0.8	561(T,P)	540	26.7	3.0×10^{-6}	572(t,P)	560	47.8
	1.6	2(T,P)	0	--	6.0×10^{-6}	24(P)	12	2.0
	Response	+				+		
C	0	21	0	--	0	26	0	--
	5.0×10^{-2}	56	35	2.7	200			
	0.2	125	104	6.0	400	30	4	1.2
	0.8	81	60	3.9	800	57	31	2.2
	3	53	32	2.5	1,600	68	42	2.6
	12	48	27	2.3	3,100	0(T)	0	--
	50	136	115	6.5	6,300	0(T)	0	--
	200	9(t)	0	--				
	Response	+				+		

TABLE 3 -- cont'd.

Waste	+S9 Dose (µg/ml)	+S9 PFU per Plate[a]	+S9 Induced PFU per Plate[b]	+S9 Fold increase	-S9 Dose (µg/ml)	-S9 PFU per Plate	-S9 Induced PFU per Plate	-S9 Fold increase
D	0	52	0	--	0	12	0	--
	800	76	24	1.5	800	19	7	1.6
	1,600	138	86	2.7	1,600	12	0	1.0
	3,100	131	79	2.5	3,100	12	0	1.0
	6,300	148	96	2.8	6,300	11	0	--
	12,500	218	166	4.2	12,500	12	0	1.0
	25,000	23(t)	0	--	25,000	0(T)	0	--
	Response	+				-		
F	0	53	0	--	0	9	0	--
	100	63	10	1.2	200	15	4	1.7
	200	78	25	1.5	800	33	24	3.7
	400	103	50	1.9	1,600	75	66	8.3
	3,100	115	62	2.2	3,100	235	226	26.1
	12,500	259	206	4.9	6,300	721	712	80.1
	25,000	3,755(P)	3,702	70.8	12,500	1,960	1,951	217.8
	50,000	416(P)	363	7.8	25,000	962	953	106.9
	100,000	0(P)	0	--	50,000	1(P)	0	--
	Response	+				+		
G	0	89	0	--	0	19	0	--
	1,250	353	264	4.0	780	20	1	1.0
	2,500	346	257	3.9	3,100	100(t)	81	5.3
	5,000	402	313	4.5	5,000	950(t)	931	50.0
	6,300	400	311	4.5	6,250	5,430(t)	5,411	285.8
	12,500	466	377	5.2	10,000	3,450(t)	3,431	181.6
	25,000	4,200(P)	4,111	47.2	20,000	2,300(T)	2,281	121.1
	40,000	1,800(P)	1,711	20.2	25,000	71(T,P)	52	3.7
	50,000	17(P)	0	--	50,000	0(T,P)	0	--
	Response	+				+		

TABLE 3 -- cont'd.

Waste	+S9 Dose (μg/ml)	+S9 PFU per Plate[a]	+S9 Induced PFU per Plate[b]	+S9 Fold increase	-S9 Dose (μg/ml)	-S9 PFU per Plate	-S9 Induced PFU per Plate	-S9 Fold increase
H	0	53	0	--	0	9	0	--
	400	33	0	--	400	10	1	1.1
	800	76	23	1.4	800	14	5	1.6
	1,600	202	149	3.8	1,600	19	10	2.1
	3,100	373	320	7.0	3,100	42	33	4.7
	6,300	591	538	11.2	6,300	64	55	7.1
	12,500	792	739	14.9	12,500	138	129	15.3
	25,000	0(P)	0	--	25,000	0(P)	0	--
	Response	+				+		
I	0	53	0	--	0	9	0	--
	800	88	35	1.7	800	11	2	1.2
	1,600	361	308	6.8	1,600	34	25	3.8
	3,100	768	715	14.5	3,100	68	59	7.6
	6,300	740	687	14.0	6,300	44	35	4.9
	12,500	936	883	17.7	12,500	27	18	3.0
	25,000	0(P)	0	--	25,000	0(P)	0	--
	Response	+				+		
J	0	62	0	--	0	12	0	--
	12	56	0	--	6	10	0	--
	50	76	14	1.2	12	13	1	1.1
	100	111	49	1.8	25	15	3	1.3
	200	173	111	2.8	50	26	14	2.2
	400	133	71	2.1	100	28(t)	16	2.3
	800	12(t)	0	--	200	10(t)	0	--
	Response	+				-		

TABLE 3 -- cont'd.

Waste	+S9 Dose (µg/ml)	+S9 PFU per Plate[a]	+S9 Induced PFU per Plate[b]	+S9 Fold increase	-S9 Dose (µg/ml)	-S9 PFU per Plate	-S9 Induced PFU per Plate	-S9 Fold increase
K	0	62	0	--	0	26	0	--
	50	69	7	1.1	200	27	1	1.0
	100	60	0	--	400	29	3	1.1
	200	66	4	1.1	800	3(t)	0	--
	400	87	25	1.4	1,600	3(t)	0	--
	800	16(t)	0	--	3,100	1(t)	0	--
Response		-				-		
L	0	62	0	--	0	12	0	--
	12	69	7	1.1	800	29	17	2.4
	400	116	54	1.9	1,600	69	57	5.8
	6,300	204	142	3.3	3,100	101	89	8.4
	12,500	464(F)	402	7.5	6,300	266	254	22.2
	25,000	2,350(F)	2,288	37.9	12,500	1,692	1,680	141.0
	50,000	0(F)	0	--	25,000	5(F)	0	--
Response		+				+		
M	0	62	0	--	0	12	0	--
	3,100	132	70	2.1	1,600	53	41	4.4
	6,300	131	69	2.1	3,100	79	67	6.6
	12,500	184(F)	122	3.0	6,300	224	212	18.7
	25,000	406(F)	344	6.5	12,500	2,085	2,073	173.8
	50,000	287(F)	225	4.6	25,000	7,590(F)	7,578	632.6
	100,000	0(F)	0	--	50,000	0(F)	0	--
Response		+				+		

TABLE 3 -- cont'd.

Waste	+S9 Dose (µg/ml)	+S9 PFU per Plate[a]	+S9 Induced PFU per Plate[b]	+S9 Fold increase	-S9 Dose (µg/ml)	-S9 PFU per Plate	-S9 Induced PFU per Plate	-S9 Fold increase
N	0	87	0	--	0	26	0	--
	37,500	107	20	1.2	37,500	28	2	1.1
	50,000	139	52	1.6	50,000	23	0	--
	75,000	135	48	1.6	75,000	23	0	--
	100,000	142	55	1.6	100,000	24	0	--
	200,000	23	0	--	200,000	16	0	--
	300,000	18	0	--	300,000	10	0	--
Response								
O	0	87	0	--	0	26	0	--
	37,500	77	0	--	37,500	12	0	--
	50,000	84	0	--	50,000	11	0	--
	75,000	94	7	1.1	75,000	12	0	--
	100,000	100	13	1.1	100,000	16	0	--
	200,000	31	0	--	200,000	10	0	--
	300,000	18	0	--	300,000	6	0	--
Response								

[a]Each value is the average of two plates. T, toxic (clear well); t, slightly toxic (slightly turbid well); P, precipitate; F, oily film on surface of medium. The positive control results were: 2-aminoanthracene (0.3mg/ml) 716 ± 288 PFU/plate; 2-nitrofluorene (150mg/ml) 400 ± 264 PFU/plate.

[b]Induced PFU/plate=observed minus background.

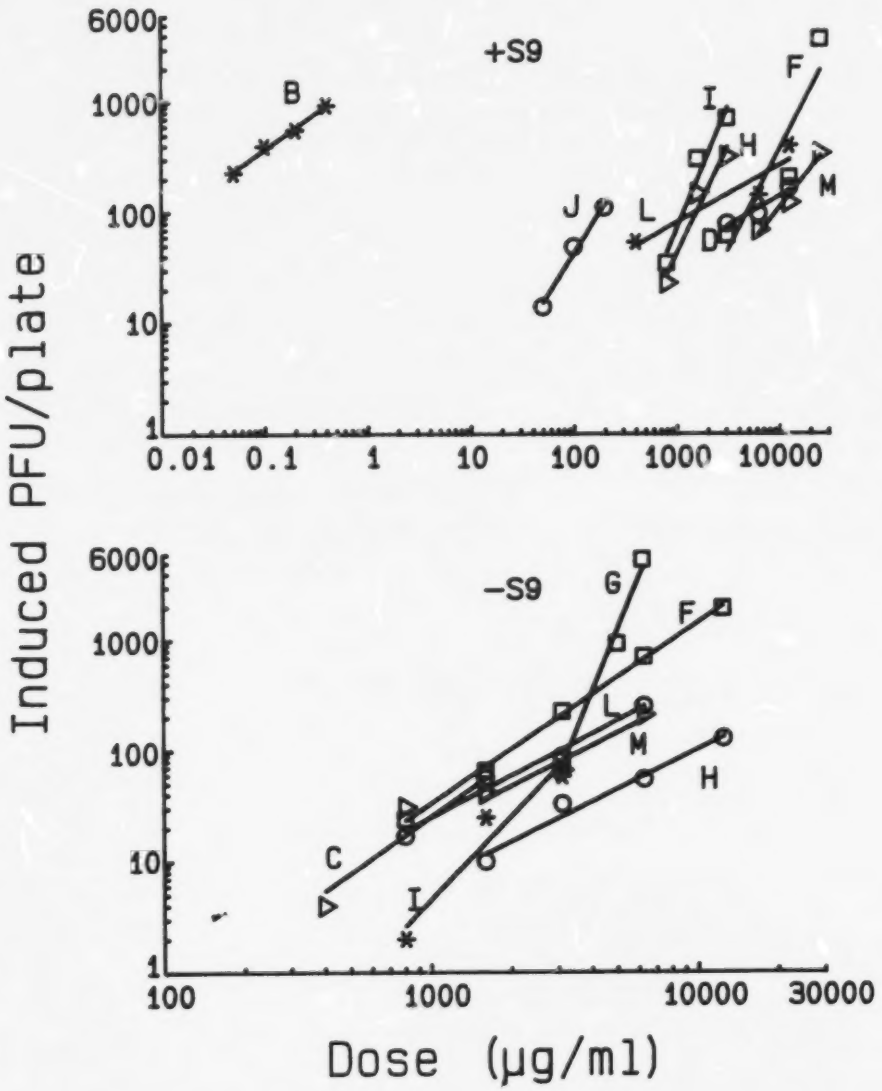

FIG. 1 -- Dose-response curves of prophage induction by hazardous waste samples in the presence and absence of S9. Only the linear portions of the dose-response curves are shown, and the correlation coefficients (r^2 values) are 0.99 for all of the curves except L (0.97) and F (0.92) in the presence of S9, and for C (0.91), M (0.98), and G (0.88) in the absence of S9.

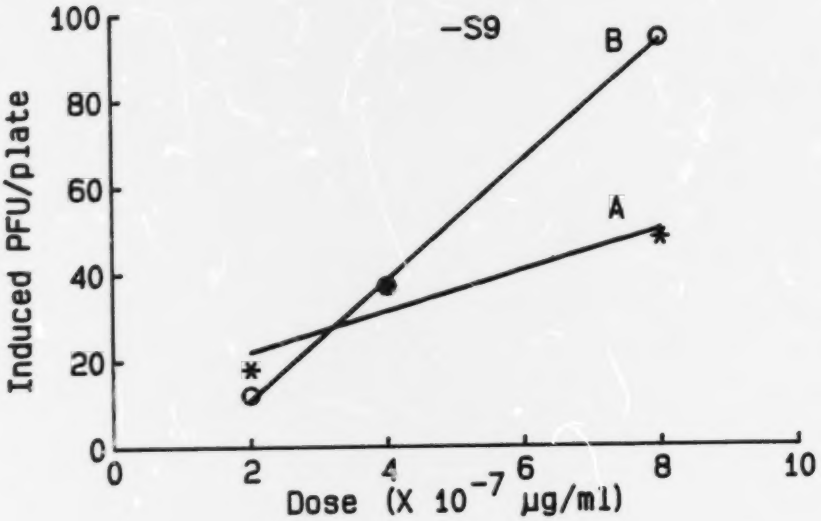

FIG. 2 -- Linear portions of the dose-response curves of prophage induction by hazardous waste samples A and B in the absence of S9. Correlation coefficients are 0.94 and 0.99 for A and B, respectively.

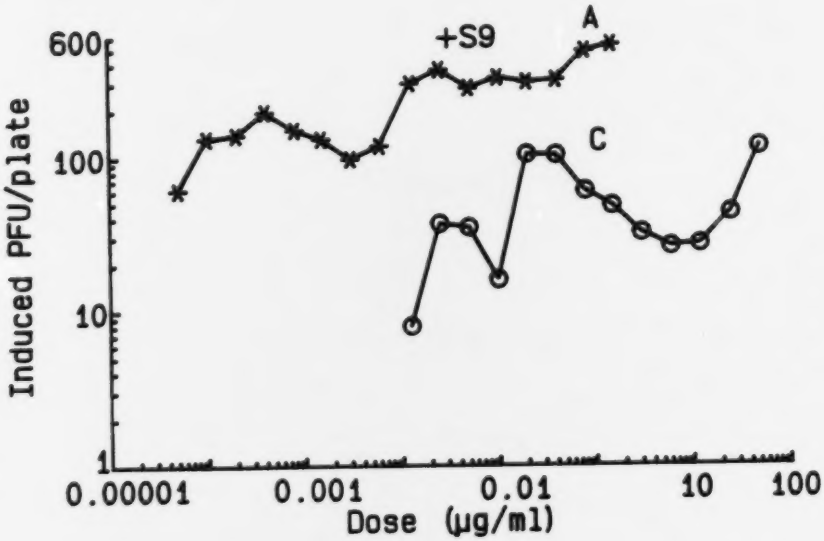

FIG. 3 -- Dose-response curves for waste samples A and C in the presence of S9.

TABLE 4 -- Relative potencies of wastes

Waste	Concentration (μg/ml) required to produce an induced PFU/plate equal to the upper limit of the 99% confidence interval based on the medium controls[a] +S9	(rank)	-S9	(rank)
B	0.03	(1)	3 X 10-7	(1)
A	+[b]		4 X 10-7	(2)
C	+		1082	(3)
J	185	(2)	-	
I	976	(3)	1888	(7)
H	1346	(4)	3355	(8)
L	3029	(5)	1200	(4)
D	6076	(6)	-	
F	6444	(7)	1490	(5)
M	9561	(8)	1638	(6)
G	+		3456	(9)

[a]This is the concentration (μg/ml) required to produce 102 induced PFU/plate (+S9) or 30 induced PFU/plate (-S9).

[b]Potency value incalculable due to nonlinear dose response (see text).

Although waste samples A and C were positive in the presence of S9, they did not produce simple linear dose responses. Instead, they produced reproducible, multimodal dose-response curves that ranged over 4 orders of magnitude (Fig. 3). In addition, waste sample G produced a nonlinear dose reponse at the doses tested in the presence of S9 (Table 3; data not plotted).

DISCUSSION

For most bioassays, the genotoxic constituents of a complex mixture must be extracted or fractionated prior to bioassay to make them compatible with the test system. However, with the Microscreen assay we were able to test the crude wastes directly, saving considerable time, effort, and expense. The unfractionated wastes posed no sterility problems, probably due to their extreme toxicity. Other wastes, however, may contain microbial contaminants that would necessitate the preparation of organic extracts.

The partial chemical characterization available for these wastes (Table 2) is more extensive than would ordinarily be available for most waste samples. However, even this degree of chemical analysis is inadequate to distinguish a genotoxic from a nongenotoxic waste or to indicate which waste might be more genotoxic than another. For example, the chemical analysis of the petrochemical and pharmaceutical waste samples (A and B, Table 2) does not necessarily suggest the remarkable cytotoxic and genotoxic potencies exhibited by these two samples compared to the other waste samples (Tables 3 and 4). Likewise, the finding that waste samples K, N, and O were not genotoxic is not readily apparent from their chemical profiles, which are

relatively indistinguishable from those of the genotoxic waste samples (Table 2). The limitations of the chemical analysis for predicting the biological effects of these wastes argue in favor of bioassays to characterize the possible hazardous nature of wastes.

The genotoxic potencies exhibited by most of the waste samples are relatively similar (Fig. 1, Table 4), which may be due to the fact that most of these wastes were composites of wastes from a variety of sources. Thus, mixtures of potent and weak wastes may result in samples with similar average potencies. Consistent with this interpretation is the fact that the two samples that have vastly different potencies from the rest (samples A and B) are not composite wastes but are from two distinct industrial sources, the petrochemical and pharmaceutical industries.

Table 5 compares the mutagenicity of these waste samples in Salmonella to the ability of these waste samples to induce prophage. All but one of the wastes that were mutagenic in Salmonella also induced prophage. This is consistent with studies of pure compounds that show that for those compounds tested, most chemicals that are mutagenic in Salmonella also induce prophage and/or the SOS response [21-25]. Thus, at least some concordance between the assays is not surprising. The inability to detect waste sample O in the phage-induction assay may have been due to a toxic or inhibitory effect of the sample on prophage production, as evidenced by a reduction in PFU/plate at concentrations above 100,000 μg/ml (Table 3).

TABLE 5 -- Comparisons of prophage induction in Escherichia coli and mutagenesis in Salmonella typhimurium

Waste	Prophage Induction in E. coli	Mutagenicity in S. typhimurium
A	+	+
B	+	-
C	+	+
D	+	-
F	+	+
G	+	+
H	+	-
I	+	-
J	+	-
K	-	-
L	+	+
M	+	+
N	-	-
O	-	+

[a]From DeMarini et al. [26]. Wastes were tested in strains TA98 and TA100. A positive response in either strain, with or without S9, was sufficient for a positive summary response reported here.

The phage-induction assay detected 5 waste samples as genotoxic that were not mutagenic in Salmonella (Table 5). As mentioned previously, the Microscreen phage-induction assay has been shown to

detect some carcinogenic metals [17], chlorinated organics [18], and solvents [19] that are not mutagenic in Salmonella [14,15]. Metals and compounds of these types are present in most of the waste samples studied here (Table 2), and the ability of some of these compounds to induce prophage may account for the detection by the phage-induction assay of the 5 additional waste samples that were not detected by the Salmonella assay. In addition, accumulating evidence indicates that phage-induction (and the SOS response in general) is a broader genetic endpoint than reverse mutation in bacteria, occurring by a variety of mechanisms and involving a number of classes of genetic damage [21,27].

Currently, the US EPA identifies wastes as hazardous based primarily on physical characteristics and chemical composition of the wastes [10,11,28]. However, the deficiencies of this definition have led EPA to consider adding health effects, including genotoxicity, to their definition of hazardous waste [29-31]. In addition to government, industry also has recognized the important role that short-term assays could play in the toxicological assessment of hazardous wastes [32,33]. In combination with chemical analysis, assessments of the biological effects of wastes could contribute valuable information regarding the hazardous nature and risk associated with exposure to industrial wastes and effluents. Our data suggest that the Microscreen phage-induction assay may provide a simple, sensitive, and inexpensive way to screen hazardous wastes for an important biological endpoint, genotoxicity.

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REFERENCES

[1] Houk, V.S. and DeMarini, D.M., "Use of the Microscreen phage-induction assay to assess the genotoxicity of 14 hazardous industrial wastes," Environmental and Molecular Mutagenesis, Vol. 11, 1988, pp. 13-29.

[2] Dietz, S., Emmet, M., DiGaetano, R., Tuttle, D., and Vincent, C., "National survey of hazardous waste generators and treatment, storage, and disposal facilities regulated under RCRA in 1981," EPA 530/SW-84-005, U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, U.S. Government Printing Office, Washington, DC, 1984.

[3] US Environmental Protection Agency, "Everybody's Problem: Hazardous Waste," Office of Water and Waste Management, Washington, DC, 1980.

[4] Grisham, J.W., "Disposal Site Health Studies," in Health Aspects of the Disposal of Waste Chemicals, Pergamon Press, New York, 1986, pp. 241-318.

[5] Clark, C.S., Meyer, C.R., Gartside, P.S., Majeti, V.A., Specker, B., Balistreri, W.F., and Elia, V.J., "Environmental health survey of drinking water contamination by leachate from a pesticide waste dump in Hardeman County, Tennessee," Archives of Environmental Health, Vol. 37, 1982, pp. 9-18.

[6] Meyer, C.R., "Liver dysfunction in residents exposed to leachate from a toxic waste dump," Environmental Health Perspectives, Vol. 48, 1983, pp. 9-13.

[7] Maugh, T.H., "Toxic waste diposal a growing problem," Science, Vol. 204, 1979, pp. 819-823.

[8] Vianna, N.J. and Polan, A.K., "Incidence of low birth weight among Love Canal residents," Science, Vol. 226, 1984, pp. 1217-1219.

[9] Warren, C.S., "Hazardous waste: An overview," in The Hazardous Waste Dilemma: Issues and Solutions, American Society of Civil Engineers, New York, 1981, pp. 5-15.

[10] Federal Register, "Hazardous waste and consolidated permit regulations," Vol. 45, No. 98, May 19, 1980. pp. 33066-33133.

[11] Greer, L.E., "Definition of hazardous waste," Hazardous Waste, Vol. 1, pp. 309-322.

[12] Ames, B.N., McCann, J., and Yamasaki, E., "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test," Mutation Research, Vol. 31, 1975, pp. 347-364.

[13] US Environmental Potection Agency, "Performance Evaluation of Full-Scale Incinerators," National Technical Information Center, Publication No. PB85-129500, 1984.

[14] Kier, L.D., Brusick, D.J., Auletta, A.E., Von Halle, E.S., Brown, M.M., Simmon, V.F., Dunkel, V., McCann, J., Mortelmans, K., Prival, M., Rao, T.K., and Ray, V., "The Salmonella typhimurium/mammalian microsomal assay. A report of the US Environmental Protection Agency Gene-Tox Program," Mutation Research, Vol. 168, 1986, pp. 69-240.

[15] Zeiger, E. and Tennant, R.W., "Mutagenesis, clastogenesis, carcinogenesis: expectations, correlations and relations." in Genetic Toxicology of Environmental Chemicals, Part B, Alan R. Liss, New York, 1986, pp. 75-84.

[16] Claxton, L.D., Stead, A.G., and Walsh, D., "An analysis by chemical class of Salmonella mutagenicity tests as predictors of animal carcinogenicity," Mutation Research, Vol. 205, 1988, pp. 197-225.

[17] Rossman, T.G., Molina, M., and Meyer, L.W., "The genetic toxicology of metal compounds: I. Induction of λ prophage in E. coli WP2s(λ)," Environmental Mutagenesis, Vol. 6, 1984, pp. 59-69.

[18] Houk, V.S. and DeMarini, D.M., "Induction of prophage lambda by chlorinated pesticides," Mutation Research, Vol. 182, 1987, pp. 193-201.

[19] Houk, V.S. and DeMarini, D.M., "Compatibility and genotoxicity of organic solvents in the Microscreen phage-induction assay," in preparation.

[20] D'Ari, R., "The SOS System," Biochimie, Vol. 67, 1985, pp. 343-347.

[21] Elespuru, R.K., "Induction of bacteriophage lambda by DNA-interacting chemicals," in Chemical Mutagens: Principles and Methods for their Detection, Vol 9, Plenum, New York, 1984, pp. 213-231.

[22] Oda, Y., Nakamura, S., Oki, I., Kato, T., and Shinagawa, H., "Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens," Mutation Research, Vol. 147, 1985, pp. 79-95.

[23] Ohta, T., Nakamura, N., Moriya, M., Shirasu, Y., and Kada, T., "The SOS- function-inducing activity of chemical mutagens in Escherichia coli," Mutation Research, Vol. 131, 1984, pp. 101-109.

[24] Quillardet, P., de Bellecombe, C., and Hofnung, M., "The SOS Chromotest, a colorimetric bacterial assay for genotoxins: validation study with 83 compounds," Mutation Research, Vol. 147, 1985, pp. 79-95.

[25] Rossman, T.G., Meyer, L.W., and Molina, M., "Induction of λ prophage as a screen for genotoxic agents," Annals of the NY Acadamy of Sciences, Vol. 463, 1986, pp. 347-348.

[26] DeMarini, D.M., Inmon, J.P., Simmons, J.E., Berman, E., Pasley, T.C., Warren, S.H., and Williams, R.W., "Mutagenicity in Salmonella of hazardous wastes and urine from rats fed these wastes," Mutation Research, Vol. 189, 1987, pp. 205-216.

[27] Hofnung, M. and Quillardet, P., "Recent developments in bacterial short-term tests for the detection of genotoxic agents," Mutagenesis, Vol. 1, 1986, pp. 319-330.

[28] Friedman, D., "An overview of selected EPA RCRA test method development and evaluation activities," in Hazardous and Industrial Solid Waste Testing: Fourth Symposium, American Society for Testing and Materials, Philadelphia, 1985, pp. 77-84.

[29] Federal Register, "Notification requirements; reportable quantity adjustments," Vol. 48, No. 102, May 25, 1983, pp. 23552-23602.

[30] Federal Register, "Hazardous waste management systems," Vol. 49, No. 32, Feb. 15, 1984, pp. 5854-5859.

[31] Federal Register, "Proposed deadlines for exposure assessment," Vol. 49, No. 227, Nov. 23, 1984, pp. 46304-46312.

[32] Barfknecht, T.R. and Naismith, R.W., "Methodology for evaluating the genotoxicity of hazardous environmental samples," Hazardous Waste, Vol. 1, 1984, pp. 93-109.

[33] Guiney, P.D., "Use of predictive toxicology methods to estimate relative risk of complex waste mixtures," Hazardous Waste and Hazardous Materials, Vol. 2, 1985, pp. 177-189.